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Evaluation of a Rapid Air Thermal Cycler for Detection of *Mycobacterium tuberculosis*

Chapin and Lauderdale (1) describe their evaluation of a rapid air thermal cycler (ATC) (Idaho Technology, Idaho Falls, ID) for the detection of *Mycobacterium tuberculosis* by PCR. We also have compared the use of the ATC to that of a more conventional heat block thermocycler (HBTC) (GeneAmp PCR System 9600; Perkin Elmer, Cheshire, England) for the direct detection of *M. tuberculosis* in clinical samples. Our findings support the general conclusions of Chapin and Lauderdale but differ significantly in regard to inhibitors.

Samples were prepared by sonication using glass beads, and we used a 123-bp sequence of IS*6110* as the target for DNA amplification, as previously described (4). However, we adapted the PCR cycling parameters for use with both the ATC and HBTC (Table 1). Since PCR of clinical samples has been hindered by inhibitors present in 3 to 20% of specimens (2, 5), each PCR mixture included an internal amplification control of 169 bp (Novocastra Laboratories Ltd., Newcastle, England) which underwent coamplification. To date, we have tested 26 clinical samples (including 24 sputum and 2 bronchoalveolar lavage samples) from 18 patients by PCR using both cyclers and compared the results with those of microscopy and culture (Table 2).

On initial testing, inhibitors (as evidenced by nonamplification of the internal control) were detected in 1 sample (3.9%) with the ATC and 10 samples (38.5%) with the HBTC. Following further purification by simple chloroform extraction (3), 2 samples still had evidence of inhibitors with the HBTC. Both of these samples were therefore nonevaluable by using the HBTC, and both were *M. tuberculosis* culture positive.

By using culture as the "gold standard" for *M. tuberculosis*, of 14 culture-positive samples studied, 10 (71.4%) were PCR positive with the ATC and 8 (57.1%) were PCR positive with the HBTC, following chloroform purification where necessary. All the culture-positive but PCR-negative samples were smear negative, suggesting a low bacillary load, although 2 of the 10 samples found to be PCR positive with the ATC were also smear negative, as was one of the 8 successfully amplified samples with the HBTC.

Of 12 culture-negative samples examined (2 of which contained *M. malmoense*), all were negative with both cyclers.

We therefore agree with Chapin and Lauderdale that the ATC is an excellent alternative to the HBTC in decreasing both overall cost and total assay time (3 versus 4.5 h). However, in our limited series, and in contrast to what Chapin and Lauderdale found, despite the smaller sample input for the ATC (1 versus 5 μl), it not only provided an assay as sensitive as the HBTC but also had fewer problems with inhibitors.

TABLE 1. PCR cycling parameters

Cycler	Initial step	Cycling parameters	No. of cycles	Cycle time
ATC	94°C, 1 min	94°C, 0 s; 50°C, 0 s; 72°C, 2 s	45	23 min
HBTC	94°C, 1 min	68°C, 30 s; 72°C, 30 s; 94°C, 15 s	15	1 h 20 min
		60°C, 30 s; 72°C, 30 s; 94°C, 15 s	15	
		60°C, 30 s; 72°C, 5 min	1	

TABLE 2. Comparison of microscopy, culture, and PCR for detection of *M. tuberculosis* in 26 clinical samples

Sample status (*n*)	ATC		HBTC	
	No. of samples PCR+	No. of samples with inhibitors	No. of samples PCR+	No. of samples with inhibitors
Smear+, culture+ (8)	8	0	7	1[a]
Smear−, culture+ (6)	2	0	1	2[a]
Smear+, culture− (2)[b]	0	0	0	0
Smear−, culture− (10)	0	1	0	7

[a] Includes one specimen containing inhibitors not removed by chloroform treatment; PCR therefore nonevaluable.
[b] Specimens grew *M. malmoense.*

REFERENCES

1. **Chapin, K., and T.-L. Lauderdale.** 1997. Evaluation of a rapid air thermal cycler for detection of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **35:**2157–2159.
2. **Forbes, B. A., and K. E. Hicks.** 1996. Substances interfering with direct detection of *Mycobacterium tuberculosis* in clinical specimens by PCR: effects of bovine serum albumin. J. Clin. Microbiol. **34:**2125–2128.
3. **Miller, N., S. G. Hernandez, and T. J. Cleary.** 1994. Evaluation of Gen-Probe amplified *Mycobacterium tuberculosis* direct test and PCR for direct detection of *Mycobacterium tuberculosis* in clinical specimens. J. Clin. Microbiol. **32:**393–397.
4. **Tan, M. F., W. C. Ng, S. H. Chan, and W. C. Tan.** 1997. Comparative usefulness of PCR in the detection of *Mycobacterium tuberculosis* in different clinical specimens. J. Med. Microbiol. **46:**164–169.
5. **Vahaboglu, H., V. Avkan, S. Dodanli, M. Uzun, I. Yildirim, and L. Mulazimoglu.** 1997. Comparison of two DNA extraction methods on inhibitory sputum samples. Clin. Microbiol. Infect. **3:**144–145.

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Authors' Reply

We are encouraged to see that other investigators have had the success with the air thermal cycler (ATC) that we have had in our laboratory. In response to the greater inhibition that Kearns et al. had with the heat block thermocycler (HBTC), we would respond with the following comments. In our laboratory, we perform chloroform purification on all of our samples. It is not clear from the letter of Kearns et al. whether they performed chloroform purification only after noting a problem with inhibition or whether further chloroform purification was performed in addition to an initial extraction. In our case, initial chloroform purification on all samples saves the majority of "headaches" of uninterpretable PCRs. However, we did not fully explain in our paper the following: if no internal control was seen with a specific specimen, the PCR was repeated with DNA samples either undiluted or in a 1:10 dilution. There were 11 samples overall for both the ATC and HBTC that

showed no internal control with the first PCR (11 of 270 [4%]). Nine of our samples with the ATC and seven with the HBTC showed no presence of inhibitors on repeat of the PCR. There were two samples with the ATC that showed inhibition on repeat and four with the HBTC that showed inhibition on repeat, <1 and 1.5%, respectively. Thus, we did not have the inhibition rate that Kearns et al. reported for initial samples (11 of 26 samples [42%]), nor did we find that the HBTC samples showed far greater inhibition than the ATC samples. Other differences between our procedure and that of Kearns et al. to which the differences in the inhibition rate may be attributed are the primer sequence being targeted and the cycling parameters. The cycling parameters for the ATC and the HBTC in our procedure are quite similar, yet those of Kearns et al. are quite different. One possible explanation for the difference between the inhibition rates is the input volume of the DNA sample and the overall ratio in the reaction mixes for the two instruments. For our samples, the ratio of DNA input to reaction mix was always 1:10 for both the ATC (1 μl in 10 μl of reaction mix) and the HBTC (5 μl in 50 μl of reaction mix). Neither the input volume nor the reaction mix ratio was clear from the letter from Kearns et al.; however, if the overall input volume of the DNA sample for the HBTC was much greater, there would likely be more inhibition with the samples.

Again, we are very excited to find others who have had success with this instrument and wish the investigators the best in their future endeavors with the ATC.

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